INTERNATIONAL STANDARD

ISO 18071

First edition
2016-07-15

Fine ceramics (advanced ceramics, advanced technical ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials under indoor lighting environment — Test method using bacteriophage Q-beta

Céramiques techniques — Détermination de l'activité antivirale des matériaux photocatalytiques semi-conducteurs dans un environnement d'éclairage intérieur — Méthode d'essai utilisant un bactériophage Q-béta

Reference number
ISO 18071:2016(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword ..... **v**
Introduction ..... **vi**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Symbols **2**
5 Principle **3**
6 Materials **3**
6.1 Strains and preparation for tests 3
6.1.1 Strains 3
6.1.2 Bacteria preparation 4
6.1.3 Bacteriophage preparation 4
6.2 Media 5
6.2.1 General 5
6.2.2 1/500 Nutrient broth (1/500 NB) 5
6.2.3 Calcium solution 5
6.2.4 LB broth with calcium 5
6.2.5 Agar powder 5
6.2.6 LB agar 5
6.2.7 Bottom agar plate (LB agar plate with calcium) 6
6.2.8 Top agar 6
6.2.9 Soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP) 6
6.2.10 Peptone saline solution 6
7 Apparatus and equipment **6**
7.1 Test equipment 6
7.2 Cover film 7
7.3 Moisture preservation glass 7
7.4 Glass tube or glass rod 7
7.5 Paper filter 7
7.6 Light source 7
7.7 UV sharp cut-off filter 8
7.7.1 Condition A (under 400 nm cut-off condition) 8
7.7.2 Condition B (under 380 nm cut-off condition) 8
7.8 Illuminance meter 8
7.9 Centrifuge 8
7.10 Sterilized syringe filter unit 8
8 Test piece **8**
9 Procedure **8**
9.1 General 8
9.2 Procedure for preparation of bacteria suspension 9
9.3 Procedure of preparation of test bacteriophage solution 9
9.4 Procedure of test for indoor-light-active photocatalytic antiviral activity 10
9.5 Indoor lighting condition 10
9.6 Measurement of titre of bacteriophage 11
10 Calculation **12**
10.1 General 12
10.2 Test requirement fulfilment validation 12
10.3 Indoor-light-active photocatalyst antiviral activity value calculation 13
10.4 Antiviral activity value calculation without indoor-light-active photocatalyst 13
11 Test report **14**

Bibliography **15**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 206, *Fine ceramics*.

Introduction

This International Standard applies to testing the antiviral activity of indoor-light-active photocatalytic ceramics and other materials produced by either coating or mixing of a light-active photocatalyst. The International Standard for testing the antibacterial activity of photocatalytic materials has been published as ISO 27447 and the International Standard for testing the antibacterial activity of indoor-light-active photocatalytic materials has been published as ISO 17094. The International Standard for determination of antiviral activity of semiconducting photocatalytic materials has also been published as ISO 18061.

The test method for cloths or textiles is not included in this International Standard because of lack of indoor-light-active photocatalytic cloths or textiles. When the indoor-light-active photocatalytic cloths or textiles with antiviral activity using indoor-light-active photocatalytic activity have been developed, a test method for indoor-light-active photocatalytic cloths or textiles will be proposed with the glass adhesion method in ISO 27447.

Fine ceramics (advanced ceramics, advanced technical ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials under indoor lighting environment — Test method using bacteriophage Q-beta

WARNING — Only personnel trained in microbiological techniques should carry out tests.

1 Scope

This International Standard specifies the determination of the antiviral activity of materials that contain indoor-light-active photocatalytic materials or have indoor-light-active photocatalytic films on the surface by a test method that measures the infectivity titre of bacteriophage Q-beta after illumination with indoor light.

NOTE In the test method, the surrogate microbe is bacteriophage Q-beta, intended as a model for influenza viruses.

This International Standard is intended for use with different kinds of indoor-light-active photocatalytic materials used in construction materials, in flat sheet, board or plate shape that are the basic forms of materials for various applications. It does not include powder, granular or porous indoor-light-active photocatalytic materials.

This International Standard is applicable to indoor-light-active photocatalytic materials produced for an antiviral applications. Other types of performance of indoor-light-active photocatalytic materials, i.e. antibacterial activity, antifungal activity, decomposition of water contaminants, self-cleaning, antifogging and air purification, are not determined by this method.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14605, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Light source for testing semiconducting photocatalytic materials used under indoor lighting environment*

ISO 27447, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials*

ISO 80000-1, *Quantities and units — Part 1: General*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
photocatalyst
substance that carries out many functions based on oxidization and reduction reactions under photoirradiation, including decomposition and removal of air and water contaminants, deodorization, and antiviral, antibacterial, antifungal, self-cleaning and antifogging actions

3.2
indoor-light-active photocatalyst
substance that reacts with artificial light source for general lighting service (i.e. indoor lighting environment)

3.3
indoor lighting environment
environment with artificial light source for general lighting service

Note 1 to entry: Does not include sunlight.

3.4
indoor-light-active photocatalytic materials
materials in which or on which the indoor-light-active photocatalyst is added by coating, impregnation, mixing, etc.

3.5
antiviral
condition decreasing the infectivity of viruses on the surface of materials

3.6
bacteriophage
type of virus which infects bacteria

Note 1 to entry: The bacteriophage used in this International Standard is Q-beta that is one of F-specific RNA phages. The bacteriophage Q-beta is not pathogenic to humans and animals, but serves to simulate Influenza viruses that are pathogenic to humans.

3.7
plaque
visible, clear area which is theoretically the result of infection and lysis of host cells by a single viable bacteriophage

3.8
indoor-light-active photocatalyst antiviral activity value
difference between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after indoor light illumination and on non-treated materials after indoor light illumination

Note 1 to entry: This value includes the decrease of number of bacteriophage plaques without indoor light illumination.

3.9
indoor-light-active photocatalyst antiviral activity value for indoor light illumination
difference between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after indoor light illumination and on photocatalytic treated materials kept in a dark place

4 Symbols

A — average of titre of bacteriophage on non-treated specimens, just after inoculation

B_D — average of titre of bacteriophage on non-treated specimens, after being kept in a dark place

$B_{F\text{-}L}$ — average of titre of bacteriophage on non-treated specimens, after indoor light illumination of intensity L under condition F

C_D — average of titre of bacteriophage on indoor-light-active photocatalytic treated specimens, after being kept in a dark place

$C_{F\text{-}L}$	average of titre of bacteriophage on indoor-light-active photocatalytic treated specimens, after indoor light illumination of intensity L under condition F
D_F	dilution factor
F	type of UV cut-off condition (condition A or condition B)
L	illuminance of indoor light
Logmax	maximum logarithmic value of titre of bacteriophage
Logmean	average logarithmic value of titre of bacteriophage for three non-treated specimens
Logmin	minimum logarithmic value of titre of bacteriophage
N	titre of bacteriophage (plaque forming unit)
V_D	antiviral activity value without indoor-light-active photocatalyst, after being kept in a dark place on a testing material
$V_{F\text{-}L}$	indoor-light-active photocatalyst antiviral activity value, after indoor light illumination at a constant intensity (F-L) on an indoor-light-active photocatalytic material
ΔV	indoor-light-active photocatalyst antiviral activity value with indoor light illumination
Z	average number of plaques in two Petri dishes

5 Principle

The test method is suitable for use in development, comparison, quality assurance, characterization, reliability and design data generation of indoor-light-active photocatalytic materials. The method is used to obtain the antiviral activity of indoor-light-active photocatalytic materials by the contact of a specimen with bacteriophage under indoor lighting condition. The method is suitable for use with flat sheet, board or plate-shaped materials.

The specimen of indoor-light-active photocatalytic treated material is inoculated with bacteriophage suspension and exposed to light for a specified period. Following exposure, the test suspension is removed and measured by the plaque forming method with *Escherichia coli* which is sensitive to bacteriophage Q-beta. The results obtained are compared with those obtained from inoculated specimens of non-photocatalytic treated material exposed to light under identical conditions to the treated material and to those obtained from inoculated specimens of both photocatalytic treated and non-treated material kept in the dark for the same period of time.

NOTE This International Standard is adapted from the common methodological concept for ISO 18061. Namely, the same apparatus without light source (see 7.6), UV sharp cut-off filter (see 7.7), and test piece size, similar procedure and calculation are adapted between this International Standard and ISO 18061. Therefore, ISO 18061 is recommended to be used as reference during actual test of this International Standard.

6 Materials

6.1 Strains and preparation for tests

6.1.1 Strains

The strains to be used in the test shall be the same as or equivalent to those described in Table 1 and supplied by an entity that is registered under the World Federation for Culture Collections or the Japan Society for Culture Collections. Aseptic manipulations using microorganisms can be performed in an appropriate safety cabinet.

Table 1 — Bacteriophage and bacteria strains to be used in test

Species	Strain number	Organization for the collection
Bacteriophage Q-beta	ATCC 23631-B1	American Type Culture Collection
	DSM 13768	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 20012	NITE Biological Resource Center
Escherichia coli	ATCC 23631	American Type Culture Collection
	DSM 5210	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 106373	NITE Biological Resource Center

NOTE ATCC23631-B1 and NBRC20012 are not strictly same, but they are from the same origin.

6.1.2 Bacteria preparation

a) Inoculate *E. coli* strain into a slant culture medium (6 ml to 10 ml of LB agar; see 6.2.6), incubate for 16 h to 24 h at (37 ± 1) °C and then store in the refrigerator at 5 °C to 10 °C.

b) Repeat subcultures within 1 month by replicating this process.

c) The slant culture shall not be used for further storing after 1 month.

d) The maximum number of subcultures from the original strain transferred by culture collection is 10.

NOTE In the case of bacteria stored in a deep freezer, the maximum number of subcultures from original strain transferred by culture collection is 10.

6.1.3 Bacteriophage preparation

a) Introduce 25 ml of LB broth with calcium (see 6.2.4) into a conical flask of 300 ml and inoculate with *E. coli* strain.

b) Incubate for (18 ± 2) h at (37 ± 1) °C while shaking at (110 ± 10) min^{-1}.

c) Pre-warm 25 ml of LB broth with calcium (see 6.2.4) in a 300 ml conical flask to 35 °C to 37 °C and inoculate with 0,025 ml of the culture prepared under b).

d) Incubate as above condition until a bacterial concentration will be reached at (2,0 ± 1,0) × 10^8 cfu/ml.

This procedure should be carried out several times to establish the relationship between turbidity measurements and colony counts. If sufficient data have been obtained, further work can be based on turbidity measurements only.

e) Inoculate the bacterial culture with Q-beta from a stock solution to a final concentration of approximately 2 × 10^7 pfu (plaque forming unit)/ml [multiplicity of infection (m.o.i.) is approximately 0,1].

f) Incubate the inoculated bacterial culture for 4 h as under b).

g) Store the culture overnight at (4 ± 2) °C.

h) Pour the culture into centrifuge tubes and centrifuge for 20 min at (4 ± 2) °C at 10 000*g*.

i) Pipette the supernatant carefully to a sterilized tube.

j) Filter bacteriophage containing supernatant suspension through a sterilized syringe filter unit to purify the bacteriophage solution.

k) Determine the titre of the bacteriophage stock solution (see 9.6) and store at (4 ± 2) °C.